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Neoacrodictys elegans gen. & sp. nov. from Hainan Province, China

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Abstract—A new anamorphic genus and species, *Neoacrodictys elegans*, is illustrated and described from dead branches of an unidentified plant in Hainan Province, China. The genus is characterized by darkly pigmented turbinate to obpyriform muriform conidia produced from monoblastic integrated terminal conidiogenous cells on macronematous unbranched conidiophores.

Key words—asexual *Ascomycota*, hyphomycetes, saprobes, taxonomy

Introduction

During our continuing surveys of dematiaceous hyphomycetes colonizing diverse plant habitats from the forests of Hainan, China, a fungus that does not match any existing genera was collected growing on unidentified dead twigs. Morphological studies and a literature review (Ellis 1971, 1976; Subramanian 1971; Matsushima 1975, 1983, 1985, 1989, 1993, 1995; Castañeda-Ruiz 1986; Castañeda-Ruiz & Kendrick 1990a,b, 1991; Wu & Zhuang 2005; Seifert & al. 2011; Ma & al. 2016, 2021; Xia & al. 2016, 2017; Xu & al. 2020a,b, 2021; Zhang & al. 2020; Niu & al. 2021) indicated that the fungus represents an undescribed genus. Molecular phylogenetic analysis confirmed its morphological identity, and the fungus is described here as a new genus and species.

Materials & methods

Isolates & morphological analysis

Samples of dead branches collected were placed in separate zip-lock plastic bags, taken to the laboratory, and then incubated at 27 °C for more than 2 weeks in an artificial climate box in 9 cm diameter plastic Petri dishes with moistened filter paper. Single-spore cultures of hyphomycetes that could not be identified on natural substrate were isolated from dead branches and incubated on potato-dextrose agar (PDA: 200 g boiled and filtered white potatoes, 20 g dextrose, 15 g agar, 1 L distilled water). All PDA plates were incubated at 25 °C for 1–2 months. The colonies were photographed using a Powershot G7X mark II digital camera. Micromorphological characters were observed using an Olympus SZX10 stereomicroscope and Olympus BX53 microscope, both fitted with Olympus DP80 high definition colour digital cameras. All fungal strains were stored in 10% sterilized glycerin at 4 °C for further studies. The specimens are deposited in the Herbarium of Plant Pathology, Shandong Agricultural University, Taian, Shandong, China (HSAUP). Ex-type cultures are deposited in the Shandong Agricultural University Culture Collection, Taian, Shandong, China (SAUCC).

DNA extraction, PCR amplification, sequencing

Genomic DNA was extracted from colonies grown on PDA, using the CTAB method (Doyle & Doyle 1990). The large subunit ribosomal RNA gene (LSU) was amplified and sequenced by using primers pairs LROR/LR5 (Vilgalys & Hester 1990, Glass & Donaldson 1995).

PCR was conducted using an Eppendorf Master Thermocycler. DNA was amplified in 25-µL volumes containing 12.5 µL Vazyme Green Taq Mix, 1 µL of each forward and reverse primer (10 µM) (Biosune), and 1 µL template genomic DNA adjusted to a total volume of 25 µL with distilled deionized water. PCR parameters were 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min, ending with a final elongation step at 72 °C for 10 min. PCR products were estimated visually by staining with GelRed after 1% agarose gel electrophoresis. Sequencing was done bi-directionally by Biosune Co. Ltd. (Shanghai, China). Consensus sequences were obtained using MEGA 7 (Kumar & al. 2016). All sequences generated in this study were deposited in GenBank (Table 1).

Sequence alignment and phylogenetic analysis

The quality of our amplified nucleotide sequences was checked and combined by MEGA 7 (Kumar & al. 2016), and reference sequences were retrieved from the National Center for Biotechnology Information (NCBI). Sequences were aligned using MAFFT 7.310 (http://mafft.cbrc.jp/alignment/server/index.html) (Katoh & al. 2019), and manually corrected using MEGA 7.

The LSU sequences were analyzed phylogenetically using Maximum-Likelihood (ML) and Bayesian Inference (BI) methods. RaxML and Bayesian analyses were run on the CIPRES Science Gateway portal (Miller & al. 2012) using RaxML 8.2.9 and MrBayes 3.2.6. Evolutionary models were calculated using MrModelTest 2.3 (Nylander

Table 1. Strains and sequences included in the phylogenetic analyses. The new sequence is set in bold.

Taxon	Voucher	GenBank LSU
Acrodictys bambusicola	CGMCC 3.18641	KX033564
Acrodictys elaeidicola	CGMCC 3.18642	KX033568
	CGMCC 3.18643	KX033569
Asterina sp.	SH-2014	KM386978
Botryotinia fuckeliana	AFTOL-ID 59	AY544651
Buelliella physciicola	Ertz 18113 (BR)	KP456147
	Ertz 19173 (BR)	KP456148
Buelliella poetschii	Ertz 18115 (BR)	KP456149
	Ertz 18116 (BR)	KP456150
Junewangia lamma	CGMCC 3.18652	KU751882
	CGMCC 3.18653	KU751883
Junewangia sphaerospora	CGMCC 3.18655	KX033572
Karschia cezannei	Cezanne-Eichler B26 (hb. Diederich)	KP456152
	Cezanne-Eichler 7453 (hb. Diederich)	KP456153
	Ertz 19186 (BR)	KP456154
Karschia talcophila	Diederich 16749 (hb. Diederich)	KP456155
Labrocarpon canariense	Ertz 16308 (BR)	KP456157
	Ertz 16907 (BR)	KP456158
'Melaspilea' lekae	Ertz 17325 (BR)	KP456162
Mycosphaerella pneumatophorae	JK5253B=AFTOL-ID 762	FJ176856
Mycosphaerellaceae sp.	SD-01	JN872645
	KH00300	GU017553
Melaspileopsis cf. *diplasiospora*	Ertz 16247 (BR)	KP456164
	Ertz 16624 (BR)	KP456165
	Ertz 16625 (BR)	KP456166
Melaspileopsis sp.	Ertz 17904 (BR)	KP456167
	Ertz 17913 (BR)	KP456168
Neoacrodictys elegans	**SAUCC H4600**	**MW907608**
Rhexoacrodictys erecta	CGMCC 3.18656	KX033555
	CGMCC 3.18657	KX033556
Rhexoacrodictys fimicola	CGMCC 3.18658	KX033553
	CGMCC 3.18660	KX033554
Stictographa lentiginosa	Ertz 17447 (BR)	KP456169
	Ertz 17570 (BR)	KP456170
	van den Boom 47621 (hb. v.d. Boom)	KP456171

2004) to select the best-fit model for each data partition according to the Akaike criterion. For ML analyses the default parameters were used and bootstrap support (BS) was calculated using the rapid bootstrapping algorithm with the automatic halt option. Bayesian analyses included two parallel runs of 5,000,000 generations, with the stop rule option and a sampling frequency set to each 1000 generations. The 50% majority rule consensus trees and posterior probability (PP) values were calculated after discarding the first 25% of the samples as burn-in. Trees were plotted in FigTree 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree) and edited with Adobe Illustrator CS 5.

Phylogenetic results

The dataset comprised 35 taxa representing 17 named species and four undetermined species including *Botryotinia fuckeliana* (AFTOL-ID 59) as outgroup. The final LSU alignment totaled 1178 characters including gaps. Of these characters, 825 were constant, 89 parsimony-uninformative, and 264 parsimony-informative. For the BI and ML analyses, the substitution model GTR+I+G for LSU was selected and incorporated into the analyses. The topology of the ML tree confirmed the tree topologies obtained from BI analyses, so only the ML tree is presented (Fig. 1). In this tree, our strain formed an independent clade.

Taxonomy

Neoacrodictys J.W. Xia & X.G. Zhang, **gen. nov.**

MB 816515

Differs from *Acrodictys* by its turbinate conidia and oblique septa that are often marked by dark bands.

Type species: *Neoacrodictys elegans* J.W. Xia & X.G. Zhang

Etymology: *Neoacrodictys* = "*neo-*" + "*acrodictys*" (Lat.), referring to its similarity to the genus *Acrodictys*.

Conidiophores macronematous, mononematous, unbranched, septate, brown to dark brown, paler towards the apex, indeterminate with flask-shaped percurrent extensions. Conidiogenous cells monoblastic, integrated, terminal, cylindrical, pale brown to brown, smooth. Conidial secession schizolytic. Conidia solitary, acrogenous, muriform, turbinate to obpyriform, euseptate, with one longitudinal and a few transverse oblique septa usually obscured by dark bands.

Neoacrodictys elegans J.W. Xia & X.G. Zhang, **sp. nov.** Fig. 2

MB 816516

Differs from *Acrodictys* spp. by its turbinate conidia and oblique septa usually obscured by dark bands.

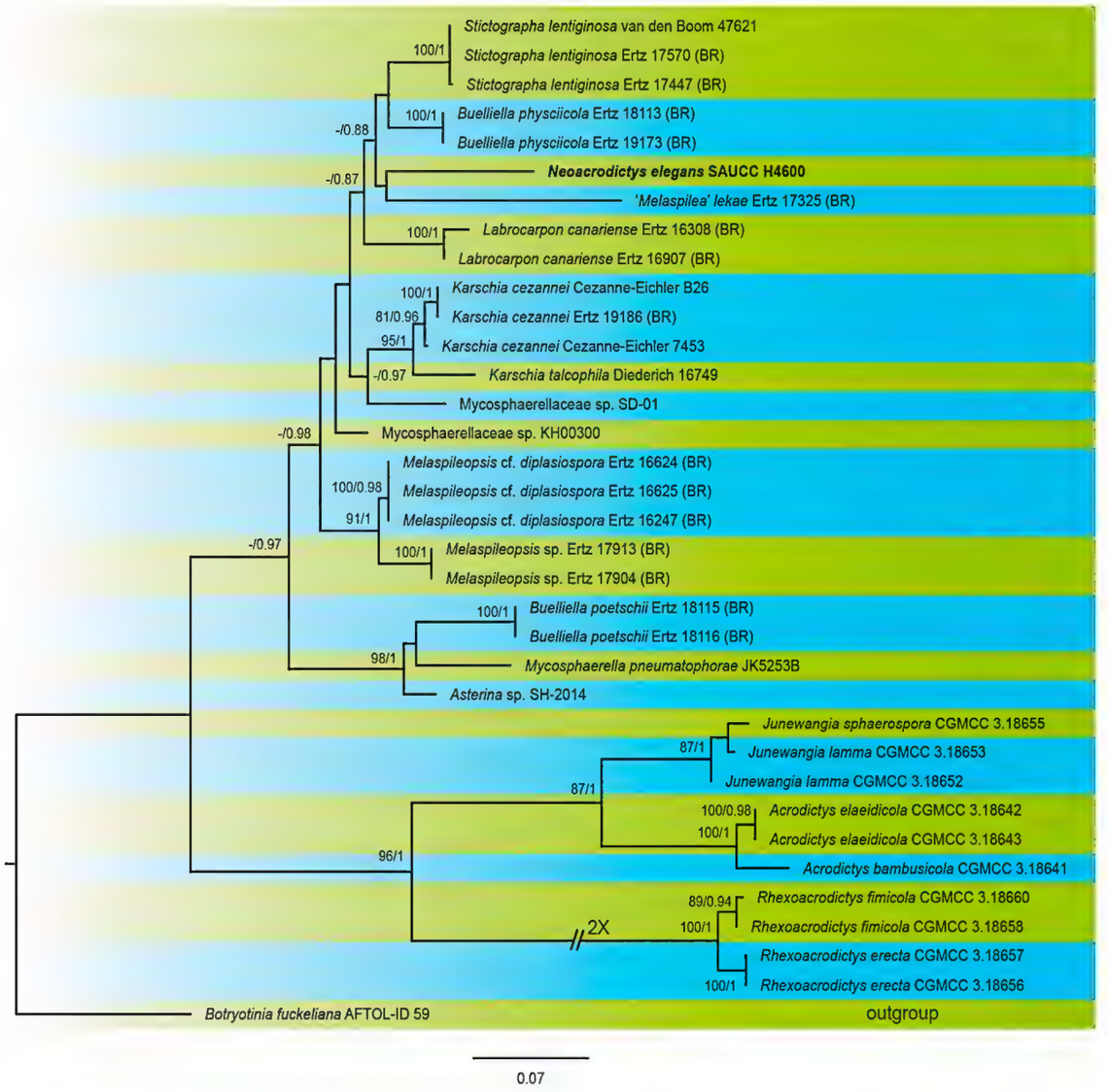

Fig. 1. Phylogram generated from RaxML analysis based on the LSU gene. Bootstrap support values are shown for ML >70% and BI >0.85. Branches shortened to fit the page are indicated by two diagonal lines accompanied by the length reduction factor. The new sequence is indicated in bold.

Type: China, Hainan Province: Ledong, Jianfengling National Forest Park, 18.70°N 108.87°E, on dead branches of an unidentified broadleaf tree, 22 April 2014, Y.R. Ma (**Holotype**, HSAUP H4600; ex-type culture, SAUCC H4600; GenBank MW907608).

Etymology: refers to its conidia that have an elegant appearance.

Colonies effuse, brown, hairy. Mycelium partly superficial, partly immersed in the substrate. Conidiophores macronematous, mononematous, erect, unbranched, straight or flexuous, thick-walled, smooth, dark brown; swollen at the base, narrower and paler toward the apex, 3–7-septate, 54–105 × 4.5–7.5 μm at the broadest part, indeterminate with 1–2 flask-shaped percurrent

extensions. Conidiogenous cells integrated, terminal, cylindrical to doliiform, subhyaline to pale brown, smooth, monoblastic; extending through the scar of the last conidium on the conidiophores. Conidia solitary, muriform, turbinate to obpyriform, 15.5–22.5 × 12–18 µm, with 1 or 2 transverse septa and 1 or 2 longitudinal or oblique septa; septa typically crossing at right angles and usually obscured by a black band. Basal cell funnel-shaped, delimited by a transverse septum, pale brown. Conidial secession schizolytic.

Discussion

Neoacrodictys demonstrates unique morphological and ontogenetic features. It is distinguished by macronematous, mononematous, cylindrical and unbranched conidiophores with holoblastic conidiogenous cells that produce darkly pigmented, muriform, turbinate to obpyriform conidia. A key to *Neoacrodictys* and morphologically similar genera is provided.

Neoacrodictys elegans resembles *Acrodictys bambusicola* M.B. Ellis, *A. elaeidicola* M.B. Ellis, and *A. malabarica* Subram. & Bhat, which differ by conidial shape (Ellis 1961, Subramanian & Bhat 1989, Xia & al. 2017). Also, the genera *Acrodictys* and *Neoacrodictys* belong in different phylogenetic clades (Fig. 1).

Key to *Neoacrodictys* and morphologically similar genera

1. Conidia bicellular ..... *Ityorhoptrum*
1. Conidia muriform 2
2. Conidia with appendages ..... *Pseudoacrodictys*
2. Conidia without appendages 3
3. Conidial secession rhexolytic ..... *Rhexoacrodictys*
3. Conidial secession schizolytic 4
4. Conidia maturing after secession ..... *Acrodictyella*
4. Conidia becoming pigmented and septate prior to secession 5
5. Conidial midpoint attached to the conidiogenous cell ..... *Coleodictyospora*
5. Conidial base attached to the conidiogenous cell 6
6. Conidiophore aseptate, comprising a single conidiogenous cell that extends percurrently after each conidial dehiscence ..... *Junewangia*
6. Conidiophores conspicuously septate, a new conidiogenous cell forming after each conidial dehiscence 7
7. Conidial basal cell inconspicuous, reduced to a short, cylindrical disk ..... *Acrodictys*
7. Conidial basal cell conspicuous, distinctly funnel-shaped and flat at the base ..... *Neoacrodictys*

Fig. 2. *Neoacrodictys elegans* (holotype, HSAUP H4600): a. Colony on PDA (surface and reverse); b. Colony on MEA (surface and reverse); c. Conidia; d–f. Conidiophores, conidiogenous cells, and conidia. Scale bars: c–f = 20 μm.

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